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Clinical value of serum miR-21 as a potential biomarker in Non-Small Cell Lung Cancer (NSCLC)

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ABSTRACT

Background: Lung cancer is one of the most common cancers in both genders worldwide. MicroRNAs (miRNAs) are small non-coding RNAs that regulate post-transcriptional gene expression acting as oncogenes or tumor suppressor genes. MicroRNAs are promising cancer biomarkers as they are easily measured, stable, and are strongly related to clinical outcomes. Recent studies have reported the role of miR-21 in many solid tumors including lung cancer. *Aim*: To evaluate the diagnostic potential of miR-21in NSCLC, correlate its level with patient's clinicopathological features in order to evaluate its prognostic value. *Methods*: This study included 50 patients with NSCLC and 10 apparently healthy matched control groups. Patients were subdivided according to their TNM classification into early-stage NSCLC (Stage I/II) (n= 6) and advanced stage (Stage III /IV) (n=44). The miR-21gene expression was measured in serum samples using quantitative real time polymerase chain reaction (qPCR). *Results*: MiR-21 gene expression was significantly increased in lung cancer patients (median: 2.58) compared to healthy controls (median: 1.45); (P=0.001). Higher expression levels were observed in stage III/IV patients' sera compared to stage I/II Patients (p<0.001). At a cut off value of >1.76, miR-21discriminated between NSCLC patients and controls with a sensitivity of 80% and specificity of 89% whereas a cut-off value of >2.4, miR-21could discriminate between patients with early and advanced lung cancer with a sensitivity and specificity of 70% and 78%; respectively. *Conclusion*: miR-21may serve as a potential non-invasive diagnostic and prognostic marker for NSCLC.

Keywords: miR-21, Non-Small Cell Lung Cancer (NSCLC), Novel biomarker.

1. INTRODUCTION

Lung cancer accounts for 11.6% of the total cancer cases worldwide and is the most common cause for cancer-related mortality (18.4% of the total cancer deaths) (Bray et al., 2018). In Egypt, lung cancer accounts for 5%-7% of all cancer types and for a quarter of all cancer-related fatalities (El-Moselhy & Elrifai, 2018). Histologically, lung cancer can be divided into small-cell lung

cancer (SCLC) and non-small-cell lung cancer (NSCLC) which is further subdivided into adenocarcinoma, squamous cell carcinoma, and large-cell carcinoma. NSCLC accounts for more than 80% of all lung cancers.

Despite advances in therapy, the 5-year survival rate remains; as low as 23.6% mainly due to late diagnosis (Hirsch et al., 2017). Traditional screening methods such as chest radiography and sputum cytology display low sensitivity and specificity in detection of lung cancer and thus, have limited clinical applications (Manser et al., 2013). Low-dose computed tomography (LDCT) has been recommended as standard screening with a mortality reduction of 20%, however, it generates a relatively large number of false-positive results (Duma et al., 2019). Therefore, exploring new biomarkers for early diagnosis of NSCLC is highly recommended.

MicroRNAs (miRNAs) are a group of small non-coding RNAs acting as important regulators in post-transcriptional gene expression. They are involved in a variety of processes considered as hallmarks of cancer such as metastasis, cell proliferation, and apoptosis. It has been reported that more than 50% of miRNAs are located at fragile sites where deletion or amplification tends to occur in human cancers (O'Brien et al., 2018). Circulating miRNAs are potential, promising cancer biomarkers. Their measurement is minimally invasive and provides fresh tumor-derived material without the complications of the traditional, invasive biopsy procedures. Being protected from endogenous RNases by vesicles or associated proteins, miRNAs are extremely stable in body fluids. Moreover, their easy assay methodology allows repeated measurements and facilitates monitoring of the disease progression, response to treatment and cancer relapse (Muller et al., 2020).

MicroRNA-21 (miRNA-21/miR-21) has been the topic of many cancer-related researches. It is encoded by the MIR21 gene located on chromosome 17q23.2 in humans. Upregulation of miR-21 has been implicated in different processes of tumorigenesis, including cell proliferation, cell survival, tumor invasion, and drug resistance (Feng and Tsao, 2016). The expression of miR-21 was found to be significantly increased in various types of malignancies such as lung, breast, ovarian, gastric and colorectal cancers. Moreover, it was negatively correlated with patient's prognosis and disease outcome (Bautista-Sánchez et al., 2020).

The aim of the current study was to evaluate the diagnostic potential of miR-21in NSCLC, and to correlate its level with patient's clinic-pathological features in order to evaluate its prognostic value.

2. METHODS

Subjects

This case-control research was carried out on 60 subjects divided into two groups, the NSCLC group and the healthy control group. The NSCLC group included 50 patients with Lung cancer selected from Ain Shams University hospital, Chest department in the period from November 2017 to June 2019. They were 42 males and 8 females, their ages ranged from 36 – 76 years with mean age of 57 years. They were diagnosed based on clinical presentation, X-ray, PET-scan, computerized axial tomography, and histopathological examination. Patients were subdivided according to their TNM stage classification into early-stage NSCLC (Stage I/II) (n= 6) and advanced stage (Stage III /IV) (n=44). The control group included 10 ages and sex matched healthy subjects. The eligibility criteria of the NSCLC patients included: de-novo patients who didn't receive chemotherapy or radiotherapy prior to sample collection. Patients who had other types of malignancy and those who received chemotherapy were excluded. The Ethics Committee of Faculty of Medicine at Ain Shams University approved the study.

Samples Collection

Three mL whole blood samples were collected in plain sterile vacutainer. After centrifugation at 4000 rpm for 20 minutes, the serum was separated, aliquoted, labelled and stored at -80^{0}C until analyzed.

Analytical Methods

Quantitative Assay of Serum MiRNA-21

a. Total RNA extraction & purification: Total RNA was extracted from serum using a miRNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The extracted RNA concentration and purity was evaluated spectrophotometrically at 260 and 280nm.

b. Reverse transcription: cDNA was synthesized by reverse transcription reaction using miScript II RT Kit (Qiagen, Hilden, Germany).

c. miR-21-3p amplification: The quantification of miR-21 levels was performed using the SYBR-Green fluorescent-based primer assay "Hs_miR-21*_1 mi Script Primer Assay targets mature miRNA", assay ID: MIMAT0004494 (Qiagen, Hilden, Germany). The miR-21 sequence is [5'CAACACCAGUCGAUGGGCUGU], the Hs_RNU6-2_11 primer assay was used as house-keeping gene for normalization. The qPCR was performed in the 5-plex Rotor Gene PCR System (Qiagen, Hilden, Germany). The 20uL reaction mixture / reaction consist of 2x Quanti Tect syber green PCR master-mix, 10x miscript universal primer, 2 uL primer

assay and 50pg- 3ng cDNA. Both targets were amplified in duplicates for each sample. The thermal protocol consists of 15 minutes for Hot Star Taq DNA Polymerase activation at 95^{0}C then 40 cycles of denaturation at 94^{0}C for 15 minutes, primer annealing for 30 seconds at 55^{0}C and extension at 70^{0}c for 30 seconds.

d. Detection and Calculation of Results: The relative expression level (fold change) for miR-21in each sample was calculated by the comparative cycle threshold 2-(ΔΔCT) method using Hs_RNU6-2 as an endogenous reference control (Schmittgen and Livak, 2008).

Statistical Analysis

Statistical analysis was performed using SPSS v.23 (Chicago, IL, USA). The mean and standard deviation were used to express descriptive statistics for quantitative parametric data. Non-parametric data were expressed as percentage for qualitative data, median and interquartile range for quantitative data. Categorical data were compared using Chi-square test while comparative analysis for gene expression between studied groups was conducted using non-parametric Mann–Whitney U test. The Receiver Operating Characteristics curve was plotted to assess the diagnostic accuracy of miR-21. The cut-off value which discriminates between different groups and the sensitivity and specificity at these levels were calculated. Significance was set at level $\leqslant 0.05$

3. RESULTS

Descriptive and comparative statistics of the demographic and clinical data of the studied groups are shown in (Table 1). There was no statistically significant difference between NSCLC and control groups regarding age, sex and smoking history (p>0.05.). The clinicopathological data of NSCLC patients are presented in (Table 2).

Table 1 Descriptive and Comparative Statistics of the Demographic Data of the Studied Groups

Parameter		Control group (n= 10)	Patients group (n= 50)	P-value
Age	Mean ± SD Range	56.80 ± 6.58 45 – 65	57.14 ± 8.22 36 – 76	0.903•
Sex	Female Male	3 (30.0%) 7 (70.0%)	8 (16.0%) 42 (84.0%)	0.296*
Smoking history	Negative Positive	4 (40.0%) 6 (60.0%)	11 (22.0%) 39 (78.0%)	0.230*

P-value >0.05: Non significant; P-value <0.05: Significant; P-value< 0.01: highly significant *Chi-square test; •Independent t-test

Table 2 Descriptive Statistics of the Clinicopathological Data of NSCLC Patients

Parameter		n (%)
Presenting Symptoms	Hemoptysis	13 (26.0%)
	Dyspnea	24 (48.0%)
	Cough	8 (16.0%)
	Chest pain	9 (18.0%)
	Weight loss	7 (14.0%)
	Hoarseness of voice	4 (8.0%)
	Headache	3 (6.0%)
	Blurring of vision	1 (2.0%)
	Accidental	2 (4.0%)
Histo-Pathological type	Adeno carcinoma	22 (44.0%)
	SCC	22 (44.0%)
	Undifferentiated NSCLC	6 (12.0%)

Stage at Presentation	II	6 (12.0%)
	III	15 (30.0%)
	IV	29 (58.0%)

Comparative statistics of the miR-21gene expression level in NSCLC cancer patients are presented in (Table 3) and (Figure 1 & 2). In our research, the level of miR-21 gene expression was upregulated by 1.7 folds in the sera of NSCLC cancer patients (median: 2.58; range: 1.8–3.0) compared to control group (median: 1.45; range: 1.04–1.9) (P=0.001). Moreover; higher expression levels were observed in stage III/IV patients' sera compared to stage I/II Patients (p<0.001).

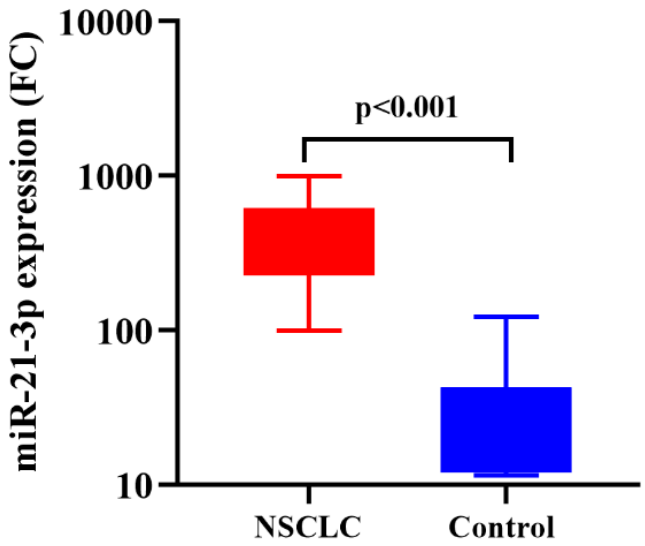

Figure 1 Boxplot representing serum expression level of miR-21 in NSCLC and healthy controls

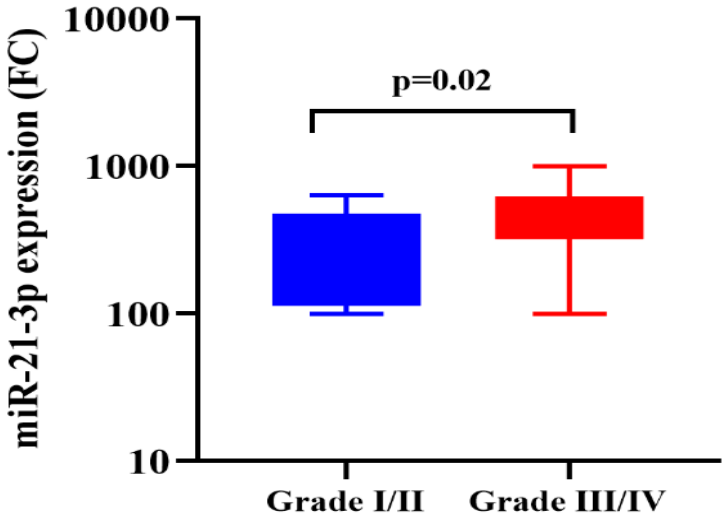

Figure 2 Boxplot representing serum expression level of miR-21 in NSCLC patients with different tumor grades

Table 3 Statistical Comparison of miR-21 Expression Levels among the Studied Groups

Group	Number of cases	Median (range)	p-value
Subjects NSCLC Control	 50 10	 2.58 (1.8–3.0) 1.45 (1.04-1.9)	0.0001*
Tumor Stage Stage II Stage III/IV	 6 44	 2.0 (1.7–2.06) 2.6 (1.9– 3.0))	0.001*

P-value >0.05: Non significant; P-value <0.05: Significant; P-value< 0.01: highly significant

* Mann Whitney test.

Receiver operating characteristic (ROC) curve was applied to assess the diagnostic performance of miR-21in NSCLC. At a cut off value of >1.76, miR-21discriminated between NSCLC patients and controls with a sensitivity of 80% and specificity of 89%. On the other hand, at an optimum cut-off value of >2.4, miR-21could discriminate between patients with early and advanced lung cancer with a sensitivity and specificity of 70% and 78%; respectively (Table 4; figures 3 & 4).

Table 4 Diagnostic Performance of MiR-21in NSCLC

	AUC	95% CI	P value	Cut-off value	Sensitivity (%)	Specificity (%)
NSCLC /Controls	0.98	0.62-0.86	0.0001	>1.76	80	89
Stage I-II/III-IV	0.78	0.54-0.82	0.006	>2.4	70	78

AUC: Area under the curve; ROC: Receiver operating Characteristics, CI: Confidence interval

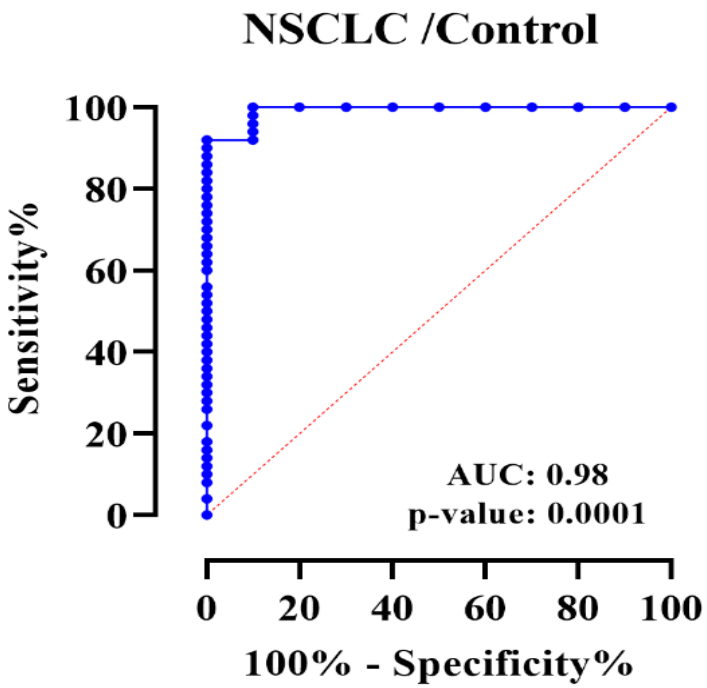

Figure 3 ROC curve illustrating the diagnostic value of miR-21 in discriminating between NSCLC patients and healthy controls.

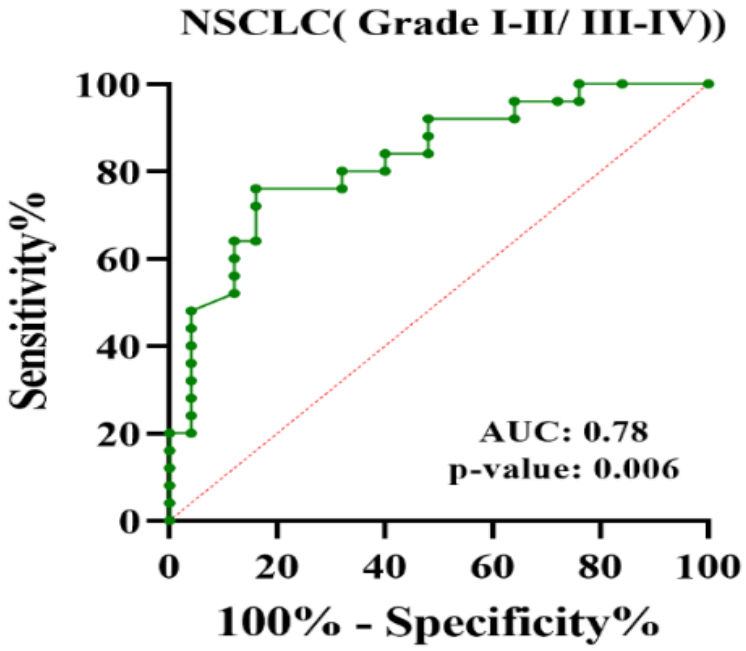

Figure 4 ROC curve illustrating the prognostic value of miR-21 in discriminating between patients with early and advanced NSCLC

4. DISCUSSION

MiRNAs are 20–24 nucleotides length non-coding RNA which control gene expression at the post-translational level. They can function as oncogenes or tumor suppressor genes and are promising candidates as molecular diagnostic and prognostic markers (Andersen & Tost, 2020). MiR-21 is an oncogenic miRNA overexpressed in multiple solid tumors including NSCLC (Bica-Pop et al., 2018). The study's objective was to evaluate the diagnostic potential of miR-21in NSCLC and to correlate its expression level with patient's clinic-pathological features in order to evaluate its prognostic value. In our study, the serum expression level of miR-21was measured in 50 NSCLC patients and 10 healthy controls by qPCR. MIR-21 gene was significantly upregulated in NSCLC than in healthy controls. At a cut off value of >1.76, miR-21 discriminated between NSCLC patients and controls with a sensitivity of 80% and specificity of 89%.

Our results agree witha previous study who compared circulating miRNA-21in 80 NSCLC patients and 80 healthy controls and revealed a significantly higher expression in patients (2.32±1.7) compared to control group (0.715 ± 0.48). The sensitivity and specificity for diagnosing NSCLC were both 80.0% at a cut-off value of 1.207 (Abu-Duhier et al., 2018). Likewise, in the study of (Qiu et al., 2018) miRNA-21 gene expression was compared in 58 patients with undifferentiated lung cancer, and 42 healthy volunteers. At a cut off level of 3.89, miRNA-21 discriminated patients from controls with a sensitivity of 86.20% and a specificity of 76.19%. Our results are also consistent with (Soliman et al., 2021) who determined the expression levels of miR-21 in 60 NSCLC patients and 40 healthy controls and reported that at a cutof point of 2.35, miRNA-21showed a sensitivity of 96.7% and a specificity of 95% in discriminating both groups.

Several studies combined miRNA-21 with other miRNAs into a test panel in an attempt to improve its diagnostic accuracy in NSCLC. Abdollahi et al., (2019) studied miRNA gene expression in 43NSCLC patients. The sensitivity and specificity of miRNA 21-were 90% and 67%, respectively; while the sensitivity and specificity of combined miRNA panel (21-5p, 638, 1481-3p, 152-3p) were 96.4% and 86.7. In another study by Sun et al., (2018) reported that in 28 patients with adenocarcinoma the sensitivity and specificity of miRNA 21 were 82.1% and 96.4%, respectively, while the sensitivity and specificity of combined miRNA panel (21 and 339-5p) were 92.9% and 92.9%. Similarly, Yang et al., (2015) enrolled152 patients with NSCLC and reported a sensitivity and specificity of 69% and 71%, respectively for miRNA-21; Combined miRNA panel (148a, 148b, 152, 21) yielded a sensitivity and specificity of 96% and 91% respectively.

In our study, serum miR-21 levels were significantly higher with advanced cancer stages (stage III/IV) compared to those with early-stage tumors (Stage I/II). At an optimum cut-off value of >2.4, miR-21could discriminate between patients with early and advanced lung cancer with a sensitivity and specificity of 70% and 78%; respectively. In concordance with our results, Wang et al., (2011) found that serum miR-21 levels in NSCLC patients were upregulated compared to healthy individuals and were correlated

with advanced TNM stages and presence of lymph node metastases. They also observed that patients with high serum miR-21 expression had significantly decreased 3-year survival rate compared with those with low serum miR-21 expression. Similar findings were also reported by (Liu et al., 2012; Zhao et al., 2015 and Zheng et al., 2018). On the other hand, (Rai et al., 2020) who included 30 NSCLC patients in his study could not find a significant distinction in expression of serum miRNA 21 in different clinical stages of the disease which they attributed to the small sample size as the majority of their patients had advanced stages (stage III: 15 patients; Stage IV: 13 patients; stage II: 2 patients).

Several studies explored miR-21 expression in NSCLC tissues compared to non-cancerous tissues. They reported that miR-21 was upregulated in malignant tissues and was significantly correlated with aggressive clinicopathological features, higher tumor grade, TNM stage and shorter overall survival (Tian et al., 2016; Li et al., 2018 and An et al., 2018). The oncogenic role of miR-21 within the cancer microenvironment could be mediated by the following mechanisms; miR-21inhibits cell apoptosis (Gou et al., 2015) and increases the cell proliferation and tumor growth rate via suppression of the tumor suppressor Phosphatase and Tensin homolog (PTEN) gene, enhancing epithelial mesenchymal transition and invasion (Zhang et al., 2010 and Marin et al., 2020). Moreover, by affecting the expression of SMAD7 protein, an essential member of the SMAD protein family, miR-21 may promote the advancement of NSCLC enhancing the invasion/migration ability (Li et al., 2018). In addition, miR-21 controls the sensitivity to radiotherapy through different mechanisms including; inhibition of PDCD4 and activation of both HIF- α and PI3K genes (Jiang et al., 2017). MiR-21 could serve as predictor to Carboplatin resistance in NSCLC patients as SMAD7 represents the direct gene target for carboplatin therapy (Lin et al., 2016).

In agreement with previous studies, in-vitro suppression of miR-21 in ovarian cancer cells significantly inhibited cell proliferation, reduced tumor invasion and promoted cell apoptosis. Similar findings were also detected in prostate cancer cell lines (Báez-Vega et al., 2016). In addition, it has been reported that suppression of miR-21 inhibited cell proliferation in breast cancer cell lines, a mechanism mediated by promoting the Leucine zipper transcription factor-like 1(LZTFL1) expression (Wang et al., 2019). Therefore, targeting the miR-21 molecular network, could be useful strategy in management of cancer.

5. CONCLUSION

Serum miR-21 levels were significantly higher in NSCLC patients compared to healthy subjects. Increased expression levels were significantly associated with advanced tumor stages highlighting the role of miR-21as potential diagnostic and prognostic marker for NSCLC. Further studies with larger sample size and patient follow up are recommended to explore the value of miR-21 in monitoring therapy and detection of relapse in NSCLC patients. A potential clinical effect of miR21-based cancer therapeutic strategies in NSCLC will be an interesting topic to explore in future studies.

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Authors' contribution

Azza Abdel Rahman Saab MD - Manuscript writing and preparation for publication, Statistical analysis and scientific review, Sharing in the molecular and the laboratory work-up of the study. Manal M Kamal Edin MD - Manuscript writing and scientific review, Sharing in the molecular and the laboratory work-up of the study. Riham Hazem Raafat MD -Clinical work-up, Data collectionand scientific review. Mohammad Sabry Alkady MD- Clinical work-up, Data collection and scientific review.

Conflict of interest

The authors declare that there are no conflicts of interests.

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Ethical approval

The study was approved by the Medical Ethics Committee of Ain Shams University (ethical approval code: FMASU MS 185/2016)

Data and materials availability

All data associated with this study are present in the paper.

REFERENCES AND NOTES

1. Abdollahi A, Rahmati S, Ghaderi B, Sigari N, Nikkhoo B, Sharifi K.A combined panel of circulating microRNA as a diagnostic tool for detection of the non-small cell lung cancer. QJM 2019; 112(10): 779-85.
2. Abu-Duhier FM, Javid J, Sughayer MA, Mir R, Albalawi T, Alauddin MS. Clinical Significance of Circulatory miRNA-21 as an Efficient Non-Invasive Biomarker for the Screening of Lung Cancer Patients. Asian Pac J Cancer Prev 2018; 19(9):2607-2611.
3. An Y, Zhang Q, Li X, Wang Z, Li Y, Tang X. Upregulated microRNA miR-21 promotes the progression of lung adenocarcinoma through inhibition of KIBRA and the Hippo signaling pathway. Biomed Pharmacother 2018; 108:1845-1855.
4. Andersen GB, Tost J. Circulating miRNAs as Biomarker in Cancer. Recent Results Cancer Res 2020; 215:277-298.
5. Báez-Vega PM, Echevarría Vargas IM, Valiyeva F, Encarnación-Rosado J, Roman A, Flores J, Marcos-Martínez MJ, Vivas-Mejía PE. Targeting miR-21-3p inhibits proliferation and invasion of ovarian cancer cells. Oncotarget 2016; 7(24):36321-36337
6. Bautista-Sánchez D, Arriaga-Canon C, Pedroza-Torres A, De La Rosa-Velázquez IA, González-Barrios R, Contreras-Espinosa L. The Promising Role of miR-21 as a Cancer Biomarker and Its Importance in RNA-Based Therapeutics. Mol Ther Nucleic Acids 2020; 5(20):409-420
7. Bica-Pop C, Cojocneanu-Petric R, Magdo L, Raduly L, Gulei D, Berindan-Neagoe I. Overview upon miR-21 in lung cancer: focus on NSCLC. Cell Mol Life Sci 2018; 75(19):3539-3551
8. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, and Jemal A. Global cancer statistics: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018; 68(6):394-424.
9. Duma N, Santana-Davila R, Molina JR. Non-Small Cell Lung Cancer: Epidemiology, Screening, Diagnosis, and Treatment. Mayo Clin Proc 2019; 94(8):1623-1640.
10. El-Moselhy EA & Elrifai, AW .Risk Factors of Lung Cancer Worldwide and in Egypt: Current Situation. Journal of Oncopathology and Clinical Research 2018; 2(2:5). 1-2
11. Feng Y and Tsao C. Emerging role of microRNA-21 in cancer. Biomedical Reports 2016; 5(4), 395-402.
12. Guo Q, Zhang H, Zhang L, He Y, Weng S, Dong Z, Wang J, Zhang P, Nao R. MicroRNA-21 regulates non-small cell lung cancer cell proliferation by affecting cell apoptosis via COX-19. Int J Clin Exp Med 2015; 15;8(6):8835-41.
13. Hirsch FR, Scagliotti GV, Mulshine JL, Kwon R, Curran WJ Jr, Wu YL, Paz-Ares L. Lung cancer: current therapies and new targeted treatments. Lancet 2017; 389(10066):299-311.
14. Jiang LP, He CY, Zhu ZT. Role of microRNA-21 in radiosensitivity in non-small cell lung cancer cells by targeting PDCD4 gene. Oncotarget 2017; 8(14):23675-23689.
15. Li X, Wu X. MiR-21-5p promotes the progression of non-small-cell lung cancer by regulating the expression of SMAD7. Onco Targets Ther 2018; (11):8445-8454.
16. Lin L, Tu HB, Wu L, Liu M, Jiang GN. MicroRNA-21 Regulates Non-Small Cell Lung Cancer Cell Invasion and Chemo-Sensitivity through SMAD7. Cell Physiol Biochem 2016;38(6):2152-62
17. Liu XG, Zhu WY, Huang YY, Ma LN, Zhou SQ, Wang YK, Zeng F, Zhou JH, Zhang YK. High expression of serum miR-21 and tumor miR-200c associated with poor prognosis in patients with lung cancer. Med Oncol 2012; 29(2):618-26.
18. Manser R, Lethaby A, Irving LB, Stone C, Byrnes G, Abramson MJ, Campbell D. Screening for lung cancer. Cochrane Database Syst Rev 2013; 6
19. Marin I, Ofek E, Bar J, Prisant N, Perelman M, Avivi C, Lavy-Shahaf G, Onn A, Katz R, Barshack I. MiR-21, EGFR and PTEN in non-small cell lung cancer: an in situ hybridisation and immunohistochemistry study. J Clin Pathol 2020; 73(10):636-641
20. Müller S, Janke F, Dietz S, Sültmann H. Circulating MicroRNAs as Potential Biomarkers for Lung Cancer. Recent Results Cancer Res 2020; 215:299-318.
21. O'Brien J, Hayder H, Zayed Y, Peng C. Overview of MicroRNA Biogenesis, Mechanisms of Actions, and Circulation. Front Endocrinol (Lausanne) 2018; 3;(9):402.
22. Qiu F, Gu WG, Li C, Nie SL, Yu F. Analysis on expression level and diagnostic value of miR-19 and miR-21 in peripheral blood of patients with undifferentiated lung cancer. Eur Rev Med Pharmacol Sci 2018; 22(23):8367-8373
23. Rai S, Garg PK, Bhatt S, Latha TK, Verma AK, Banerjee BD, Singh MP. The diagnostic role of microRNA 21 in patients with nonsmall cell lung cancer: An exploratory study. Lung India 2020; 37(6):501-505.
24. Schmittgen TD and Livak KJ. Analyzing real-time PCR data by the comparative C (T) method. Nat Protoc 2008; 3(6):1101-1108
25. Soliman SE, Abdelaleem AH, Alhanafy AM, Ibrahem RA, Elhaded ASA, Assar MFA. Circulating miR-21-5p and miR-126-3p: diagnostic, prognostic value, and multivariate analysis in non-small-cell lung cancer. Mol Biol Rep 2021; 48(3):2543-2552.

26. Sun Y, Mei H, Xu C, Tang H, Wei W. Circulating microRNA-339-5p and -21 in plasma as an early detection predictors of lung adenocarcinoma. Pathol Res Pract 2018; 214(1):119-125.
27. Tian L, Shan W, Zhang Y, Lv X, Li X, Wei C. Up-Regulation of miR-21 Expression Predicate Advanced Clinicopathological Features and Poor Prognosis in Patients with Non-Small Cell Lung Cancer. Pathol Oncol Res 2016; 22(1):161-7.
28. Wang H, Tan Z, Hu H, Liu H, Wu T, Zheng C, Wang X, Luo Z, Wang J, Liu S, Lu Z, Tu J. microRNA-21 promotes breast cancer proliferation and metastasis by targeting LZTFL1. BMC Cancer 2019; 19(1):738
29. Wang ZX, Bian HB, Wang JR, Cheng ZX, Wang KM, De W. Prognostic significance of serum miRNA-21 expression in human non-small cell lung cancer. J Surg Oncol 2011; 104(7):847-51.
30. Yang JS, Li BJ, Lu HW, Chen Y, Lu C, Zhu RX, Liu SH, Yi QT, Li J, Song CH. Serum miR-152, miR-148a, miR-148b, and miR-21 as novel biomarkers in non-small cell lung cancer screening. Tumour Biol 2015; 36(4):3035-42.
31. Zhang JG, Wang JJ, Zhao F, Liu Q, Jiang K, Yang GH. MicroRNA-21 (miR-21) represses tumor suppressor PTEN and promotes growth and invasion in non-small cell lung cancer (NSCLC). Clin Chem Acta 2010; 411(11–12):846–852
32. Zhao W, Zhao JJ, Zhang L, Xu QF, Zhao YM, Shi XY, Xu AG. Serum miR-21 level: a potential diagnostic and prognostic biomarker for non-small cell lung cancer. Int J Clin Exp Med 2015; 8(9):14759-63.
33. Zheng W, Zhao J, Tao Y, Guo M, Zhou YA, Chen C, Nalin Q and Xu L. microrna-21: a promising biomarker for the prognosis and diagnosis of non-small cell lung cancer (review). oncology letters 2018; 16(3):2777-2782.